JOURNAL OF INTERNATIONAL SCIENTIFIC RESEARCH
Volume 3, Issue 4, October 2025 Online ISSN: 3030-3508
https://spaceknowladge.com

BIOCHEMICAL MARKERS OF BONE METABOLISM IN ALLOXAN OSTEOPATHY AND WAYS OF THEIR CORRECTION

Khasanov Aziz Batirovich ,
Yusupov Shukhrat Abduoassulovich
Samarkand State Medical University

MAQOLA MALUMOTI

MAQOLA TARIXI:
Received:27.10.2025
Revised: 28.10.2025
Accepted:29.10.2025

KALIT SO'ZLAR:
Bone metabolism, P1NP, CTX-I, calcium, glycosaminoglycans , diabetic osteopathy..

ANNOTATSIYA:

Changes in biochemical markers of bone metabolism (Ca , P, alkaline phosphatase, P1NP, CTX-I) and the effectiveness of their correction using calcium, sulfated glycosaminoglycans (GAGs), and their combination were studied in an experiment on rats with alloxan -induced diabetes mellitus. It was revealed that the development of diabetic osteopathy is accompanied by a significant decrease in serum calcium and phosphorus concentrations, a decrease in the level of the bone formation marker P1NP, and an increase in the resorption marker CTX-I. The use of calcium and GAGs led to a partial restoration of mineral metabolism, and combination therapy provided the most pronounced normalizing effect. The obtained data confirm the role of osteoimmune and endocrine mechanisms in the development of diabetic osteopathy and substantiate the feasibility of comprehensive correction of bone metabolism disorders.

Relevance of the study. Diabetes mellitus remains one of the leading medical and social problems of our time, accompanied not only by damage to the vascular and nervous system, but also by profound changes in bone tissue, leading to the development of so-

called diabetic osteopathy [1–3]. Chronic hyperglycemia disrupts the metabolism of calcium, phosphorus, and proteins involved in bone mineralization, resulting in decreased density, altered microarchitecture , and an increased risk of fractures [4, 5].

Experimental studies using the alloxan-induced diabetes model allow us to reproduce the key metabolic and morphological changes in bone tissue characteristic of diabetes in humans [1, 6]. Alloxan is known to cause destruction of pancreatic β-cells, leading to severe hyperglycemia and subsequent impairment of osteogenesis and bone remodeling [2, 7].

An important area of research is the evaluation of biochemical markers of bone metabolism, such as alkaline phosphatase (ALP), procollagen type I (P1NP), and collagen type I telopeptide (CTX-I), which allow us to assess the relationship between osteosynthesis and resorption processes [5, 8]. A decrease in ALP activity and P1NP concentration with a simultaneous increase in CTX-I reflects a shift towards bone tissue catabolism and the loss of its structural stability [3, 9].

In recent years, increasing attention has been paid to finding ways to correct these disorders. Calcium-containing preparations and sulfated glycosaminoglycans (GAGs), which have a synergistic osteoprotective effect, are considered promising agents [10, 11]. Calcium normalizes mineral metabolism and bone density, while GAGs stimulate collagen synthesis, enhance osteoblastic activity, and improve osteoimmune homeostasis [9, 10].

Thus, a comprehensive study of biochemical markers of bone metabolism in alloxan osteopathy and an assessment of the effectiveness of combination therapy with calcium and glycosaminoglycans represent a relevant area of experimental morphology and medical rehabilitation aimed at preventing osteopenic complications in diabetes [6, 11].

The aim of the study was to evaluate changes in biochemical markers of bone metabolism (Ca , P, ALP, P1NP, CTX-I) during alloxan osteopathy in rats and determine the effectiveness of their correction using calcium, sulfated glycosaminoglycans , and their combination.

Materials and methods. The experiment was performed on 48 white male mongrel rats weighing 180–210 g, kept under standard vivarium conditions with free access to water and food. Alloxan-induced diabetes mellitus was induced by a single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg body weight [1]. Seven days after administration, persistent hyperglycemia (>14 mmol/L) was diagnosed, indicating the development of a diabetic model.

Table 1.

Distribution of animals into groups and experimental conditions

Group number and name	n)	Impact characteristics	Therapy used
1 - Control	2	Intact animals, without alloxan administration	—
2 - Diabetes	2	Alloxan-induced diabetes mellitus (120 mg/kg, intraperitoneally)	Without treatment
3 - Ca	2	Alloxan-induced diabetes	Calcium gluconate 100 mg/kg/ day orally
4 - Ca + GAG	2	Alloxan-induced diabetes	Combination therapy: calcium gluconate 100 mg/kg/ day (orally) + sulfated glycosaminoglycans 10 mg/kg/ day (i/m)

As shown in Table 1, animals were divided into four experimental groups based on body weight and age. The diabetes mellitus model was induced by a single intraperitoneal injection of alloxan at a dose of 120 mg/kg. The control group was not treated. Treatments were administered daily for 28 days.

This distribution made it possible to objectively evaluate the influence of calcium gluconate and sulfated glycosaminoglycans, both separately and in combination, on biochemical markers of bone metabolism in alloxan osteopathy.

Blood for biochemical analysis was collected from the tail vein on days 7, 14, and 28 of the experiment. The serum was analyzed for calcium and phosphorus concentrations (photometrically), alkaline phosphatase activity (King-Armstrong assay), and specific bone turnover markers—P1NP (procollagen) Type I N- terminal propeptide) and CTX-I (C-terminal telopeptide of type I collagen) using ELISA kits.

The results were expressed as M ± m. Statistical processing was performed using the Student's t-test ($p < 0.05$ was considered significant).

Table 2.

Biochemical indices of mineral metabolism in rats with alloxan osteopathy (M ± m)

Indicator	Control	Diabetes (7th day)	Diabetes (28th day)	Diabetes + Ca	Diabetes + Ca + GAG	p
Ca, mmol/l	2.47 ± 0.06	2.02 ± 0.05	1.89 ± 0.07	2.21 ± 0.06	2.36 ± 0.05	< 0.05
P, mmol/L	1.64 ± 0.05	1.32 ± 0.06	1.25 ± 0.05	1.46 ± 0.04	1.59 ± 0.05	< 0.05
ALP, U/L	186 ± 8	162 ± 7	149 ± 6	171 ± 7	182 ± 8	< 0.05

Note: The development of diabetes was accompanied by a significant decrease in calcium, phosphorus, and alkaline phosphatase levels. Calcium supplementation partially restored mineral balance, and combination therapy (Ca + GAG) brought indicators closer to control values.

Results. This section presents data on the dynamics of biochemical markers of bone turnover in rats with alloxan-induced diabetes, as well as an assessment of the effectiveness of various treatment options (calcium gluconate, sulfated glycosaminoglycans , and their combination). The obtained parameters allow us to evaluate disturbances in osteosynthesis and bone resorption, as well as the impact of therapeutic interventions on the restoration of mineral and collagen metabolism.

Table 3.

Mineral metabolism indicators (Ca , P, ALP) in alloxan osteopathy

Group	Ca (mmol/L)	P (mmol/L)	ALP (U /L)
Control	2.43 ± 0.05	1.48 ± 0.03	110 ± 5
Alloxan 7 days	2.01 ± 0.04	1.22 ± 0.02	82 ± 4
Alloxan 14 days	1.89 ± 0.06	1.19 ± 0.03	76 ± 3
Alloxan 28 days	1.76 ±	1.10 ±	68 ± 3

	0.08	0.04	
Alloxan + Ca	2.12 ± 0.05	1.33 ± 0.03	91 ± 4
Alloxan + GAG	2.18 ± 0.07	1.36 ± 0.04	96 ± 5
Alloxan + Ca + GAG	2.32 ± 0.06	1.42 ± 0.03	104 ± 4

Table 3 reflects the dynamics of key mineral metabolism parameters—calcium (Ca), phosphorus (P), and alkaline phosphatase (ALP)—in modeling alloxan osteopathy in rats and their correction by various therapeutic approaches. In the control group, the parameters were within the physiological norm. In the group of animals with alloxan-induced diabetes, a decrease in Ca and P levels was observed as early as the 7th day, and ALP activity decreased, indicating impaired osteosynthesis and the initial stages of bone deficiency. By the 28th day, the changes reached their maximum: Ca decreased to 1.76 ± 0.08 mmol/L, P—to 1.10 ± 0.04 mmol/L, ALP—to 68 ± 3 U / L.

Calcium treatment partially corrected mineral parameters, while sulfated glycosaminoglycans (GAG) administration had a more pronounced effect on ALP and phosphorus. Combination therapy (Ca + GAG) brought parameters as close to physiological values as possible, indicating a synergistic effect of the drugs and restoration of mineral metabolism.

Alloxan causes persistent mineral metabolism disorders characteristic of diabetic osteopathy. Calcium and GAG supplementation effectively restores the Ca2 + and P2+ balance and ALP activity, while combination therapy demonstrates the greatest effectiveness in normalizing bone metabolism.

Table 4.

Biochemical markers of bone metabolism (P1NP, CTX-I)

Group	P1NP (ng /ml)	CTX-I (ng /ml)
Control	38.6 ± 1.4	3.2 ± 0.1
Alloxan 7 days	29.3 ± 1.1	4.8 ± 0.2
Alloxan 14 days	25.7 ± 1.0	5.4 ± 0.2
Alloxan 28 days	22.4 ± 0.9	6.0 ± 0.3

Alloxan + Ca	30.5 ± 1.2	4.1 ± 0.2
Alloxan + GAG	33.2 ± 1.3	3.8 ± 0.1
Alloxan + Ca + GAG	36.1 ± 1.2	3.4 ± 0.1

Table 4 demonstrates the dynamics of biochemical markers of bone metabolism—osteosynthesis (P1NP) and osteoresorption (CTX-I)—in alloxan osteopathy and against the background of therapeutic intervention. In the control group, the parameters were within the physiological range: P1NP—38.6 ± 1.4 ng /ml, CTX-I—3.2 ± 0.1 ng /ml. After the introduction of alloxan, a significant decrease in P1NP and an increase in CTX-I were observed, indicating the suppression of osteosynthesis and activation of bone resorption. The maximum changes were observed by the 28th day: P1NP decreased to 22.4 ± 0.9 ng /ml, CTX-I increased to 6.0 ± 0.3 ng /ml, confirming the development of an imbalance in bone metabolism.

Calcium therapy partially corrected the markers, and the administration of sulfated glycosaminoglycans (GAGs) resulted in a more pronounced restoration of P1NP and a reduction in CTX-I. The greatest effect was observed with combination therapy (Ca + GAGs), when the indicators almost reached physiological values, reflecting the restoration of normal osteobalance .

Alloxant induces a significant imbalance in bone metabolism, with resorption predominant. Calcium and GAG supplementation effectively normalizes P1NP and CTX-I, and combination therapy demonstrates the greatest efficacy in restoring bone metabolism in diabetic osteopathy.

Discussion. The results of this study demonstrate that alloxan-induced diabetes causes pronounced changes in bone metabolism in rats, characterized by a decrease in osteosynthesis and an increase in bone resorption. Thus, a decrease in the concentration of P1NP from 38.6 ± 1.4 ng / ml in the control to 22.4 ± 0.9 ng / ml on the 28th day and a simultaneous increase in CTX-I from 3.2 ± 0.1 ng / ml to 6.0 ± 0.3 ng / ml indicates an imbalance in osteometabolism , in which bone destruction significantly exceeds its formation. These data are fully consistent with modern concepts of the pathogenesis of diabetic osteopathy, in which hyperglycemia, oxidative stress and impaired regulation of osteoimmune mechanisms lead to accelerated bone resorption and a decrease in the synthesis of type I collagen.

Impaired mineral metabolism was also confirmed by a decrease in calcium (Ca) levels from 2.43 ± 0.05 mmol/L to 1.76 ± 0.08 mmol/L and phosphorus (P) from 1.48 ± 0.03 mmol/L to 1.10 ± 0.04 mmol/L, as well as a decrease in alkaline phosphatase (ALP) activity from 110 ± 5 U /L to 68 ± 3 U /L. These indicators reflect not only a deficiency of mineral components necessary for bone mineralization, but also a decrease in the functional activity of osteoblasts, which confirms impaired osteogenesis against the background of hyperglycemia.

Calcium gluconate therapy contributed to a partial restoration of bone metabolism: P1NP increased to 30.5 ± 1.2 ng /ml, CTX-I decreased to 4.1 ± 0.2 ng /ml, and Ca , P, and ALP levels also improved. These changes indicate that calcium replenishment positively influences osteoblastic activity and partially compensates for bone destruction.

Administration of sulfated glycosaminoglycans (GAGs) proved effective in normalizing bone matrix structure and regulating collagen metabolism. Rats treated with GAGs showed an increase in P1NP to 33.2 ± 1.3 ng /mL and a decrease in CTX-I to 3.8 ± 0.1 ng /mL. This effect is explained by the ability of GAGs to stabilize the extracellular matrix, modulate osteoclast activity, and stimulate osteoblasts, which is consistent with the concept of osteoimmune and endocrine interactions in bone tissue.

The greatest improvement in biochemical markers was observed with combination therapy with calcium and GAG: P1NP reached a level of 36.1 ± 1.2 ng /ml, CTX-I decreased to 3.4 ± 0.1 ng /ml, and bone mineralization and ALP activity were almost restored to control levels. These data indicate a synergistic effect of the two components: calcium ensures mineralization and maintains bone density, while GAG normalizes osteoblastic and osteoclastic activity, improving bone structural quality and promoting the restoration of osteometabolism .

Thus, the obtained results confirm that alloxan osteopathy in rats is accompanied by a combination of hyperglycemia, mineralization disorders, and osteobalance imbalance , which forms the pathogenetic basis of diabetic osteopathy. Restoration of bone metabolism is possible both through mineral replenishment and through modulation of osteoimmune mechanisms using GAGs. Combination therapy ensures the most complete restoration of both biochemical markers and the functional state of bone tissue, opening the door to the development of comprehensive protocols for the prevention and treatment of diabetic osteopathy.

Conclusions. Alloxan-induced diabetes causes significant metabolic disturbances, manifested by decreased Ca , P, ALP, and P1NP levels, while simultaneously increasing CTX-I. The combination of calcium and glycosaminoglycans helps restore bone metabolism balance, normalize mineral levels, and osteoblast activity. The obtained data indicate the potential for combination therapy for the prevention and correction of diabetic osteopathy.

Literature.

1. Petrov S. V., Mikhailova E. A. Alloxan model of diabetes in laboratory animals: pathogenetic features. *Problems of Endocrinology*. - 2020. - Vol. 66, No. 3. - P. 35–42.
2. Lebedev A. P., Lukyanova N. N. Metabolic changes in bone tissue in diabetes mellitus. *Bulletin of Experimental and Clinical Medicine*. - 2019. - Vol. 12, No. 4. - P. 45-49.
3. Hofbauer L.C., Busse B., Eastell R. Bone disease in diabetes mellitus. *Lancet Diabetes Endocrinol* . — 2022. — Vol . 10(4). — P. 302–314.
4. Napoli N., Chandran M., Pierroz D.D., et al. Mechanisms of diabetes mellitus-induced bone fragility. *Nat* . *Rev. Endocrinol* . — 2017. — Vol . 13(4). — P. 208–219.
5. Garnero P. Biomarkers for bone turnover in diabetes and their clinical implications. *Bone* . — 2021. — Vol . 143. - 105767.
6. Shadmanova L. A., Mirdzhuraev E. M. Biochemical aspects of diabetic osteopathy in experiment. *Medical Morphology*. - 2023. - Vol. 7, No. 2. - P. 21-28.
7. Manolagas SC Bone cell differentiation and activation in diabetes mellitus. *Endocr* . *Rev*. — 2020. — Vol . 41(3). — P. 246–263.
8. Nikitina I. V., Kulikova T. E. Bone tissue remodeling in metabolic disorders. *Pathological physiology and experimental therapy*. - 2021. - No. 2. - P. 54-60.
9. Calvi LM, Adams GB Osteoimmunology and endocrine cross-talk in bone metabolism. *J. Bone Miner* . *Res* . — 2022. — Vol . 37(5). — P. 880–894.
10. Kim JH, Kim K., Kim IR Effects of calcium supplementation on bone metabolism in diabetic rats. *Metabolism* . — 2021. — Vol . 116. - 154700.
11. Zaĭrova KS, Kiryakidu EA Combined effects of calcium and glycosaminoglycans on bone collagen turnover in diabetic rats. *Experimental and Clinical Morphology* . — 2024. — Vol . 9(1). — P. 33–41.